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EXPERIMENTS ON THE BACTERICIDE INFLUENCE OF THE ANIMAL BODY

by

George Nuttall.-

In recent years Metschnidoff has sought to substantiate the concept that protection of the animal body against infectious diseases and the development of acquired immunity comes about as a result of the activity of phagocytes,that is to say, cells that take up or consume the penetrating bacteria and destroy them.

The experimental works with which Metschnikoff seeks to support his theory c onsist (if we disregard observations on Daphnia) essentially of experiments with anthrax bacilli on frogs and rabbits, and studies on the reciprocal behavior of anthrax bacilli and leukocytes in various animals outside the body, made on a heatable stand.

In his experiments on the frog Metschnikoff found that pieces of organs taken from animals having died of anthrax no longer contained any live anthrax bacilli after they had remained for a few days under the skin of a live frog. The pieces were then in no way virulent to susceptible animals. Microscopic examination always revealed numerous leukocytes at the periphery of the piece and the anthrax bacilli were contained in these, for the most part degenerated and seemingly dead.

The experiments on warm-blooded animals were made on rabbits. Metschnikoff placed cultures of weakened anthrax bacilli contained in glass tubes under the skin of the ear of the experimental animals, then broke the tubes and was able to establish by means of microscopic preparations that the pus produced contained ma ssive amounts of leukocytes that had taken up anthrax bacilli. Virulent bacilli were not taken up by susceptible animals, while they were taken up in abundant quantities by immune animals.

Similarly, by direct observation on the heatable stand, Metschnikoff was able to demonstrate the uptake of anthrax bacilli by leukocytes and their destruction in the cells.

All these experiments, which apparently concordantly support the significance of phagocytes, raise a number of objections, however, which are described in detail in the work of Dr. Bitter published next to this. I shall merely single out the most striking from among these, namely that so far it has not yet been established that the uptake of the bacilli in the viable state is carried out by phagocytes, even though such proof must decidedly be furnished before we can recognize the role of phagocytes as that of protecting the body against infectious agents.

In view of the great importance of the problem of the cause of acquired immunity on the one hand, and Metschnikoff's still insufficient proof on the other, a check of Metschnikoff's results seemed urgently desirable, and I have therefore complied with the urging of Prof. Fluegge to repeat these experiments.

My object first of all was to establish whether the phagocytes really take up live bacilli and whether they alone were capable of destroying bacilli. It was found that the uptake into cells was limited only to a certain fraction of bacilli, while on the other hand another fraction was destroyed without contact with the cells, through some other influences exerted in the live body, and therefore the functional significance of phagocytes must become a matter of doubt and it is indeed possible that they are only capable of taking up bacilli that are already degenerated as a result of some other influences.

I first of all repeated Metschnikoff,'s experiments on the frog; next I made a few comparative experiments with weakened and virulent anthrax bacilli on the rabbit ear; finally I studied the observations on the relationships between leukocytes and bacilli on the heatable stand on a larger scale.

The experiments for the most part were made in the winter of 1886-87 in Gottingen, with a few supplemental ones at the beginning of the winter of 1887-88, carried out in Breslau.

I. EXPERIMENTS ON FROGS

A. Experimental Procedure

Small pieces of lung, roughly the size of half a lentil, from mice having just died of virulent anthrax, were placed under the dorsal skin

of frogs and left there for varying periods of time. The introduction of the pieces in one series (table I) took place so far as possible under aseptic conditions. In another series (table II) the skin of the experimental animals was not disinfected.

The frogs inoculated in the manner described were kept at a temperature of around 16°C by day and one that did not drop below 10°C by night.

After the inoculated piece had remained under the skin of the animal for varying lengths of time it was removed and used for microscopic examination. The piece had lost its natural color within a few days and was surrounded and permeated by a gelatinous, greyish-yellow exudate. A small amount of it was mixed on a slide with a drop of sterilized physiological saline. Next a cover glass was placed on it and to prevent drying of the preparation, this was surrounded by a ring of paraffin. These preparations allow prolonged study and have the advantage that the leukocytes keep their mobility in them for a period of time, so that any possible uptake of bacilli can very easily be observed. At the same time a few dry preparations were also made in the usual manner, stained with methylene blue and examined after embedding in Canada balsam. Preparations carefully made in this manner, contrary to Metschnikoff's claims, in my experience, do not damage either the bacilli or the leukocytes in any appreciable way in their form relationships.

The rest of the inoculum was used in part for plate cultures and in part to inoculate mice.

B. Experimental Results

In the preparations made from the piece used for inoculation there were numerous leukocytes in all cases, with multiple or lobulated nuclei and also cells with a large, pale nucleus. In fresh preparations a large part of the cells revealed amoeboid movement. In order to obtain reasonably accurate indications of the percentual ratio between bacilli possibly taken up and those not taken up in the preparations, counts were attempted. These took place by counting the bacilli that were present in several successive visual fields until a figure of 200 was reached. It was noted how many of these 200 bacilli lay free, and how many inside cells. These counts were repeated a few times at various points of the preparation and the mean of the results obtained in this manner was calculated.

As is shown by the tables on the previous pages, the uptake of bacilli by leukocytes having migrated into the inoculated piece appears

to proceed somewhat slowly. After the inoculated piece of tissue had been under the frog's skin for 16 hours, despite the abundance of leukocytes, none could be found that contained bacilli. Metschnikoff, however, claims having encountered an abundance of cells containing bacilli as early as 12 to 15 hours later.

On the other hand, after 22 hours a fairly appreciable uptake had taken place. In one case 27% of all bacilli present were observed to be within cells, although in others only 1%.

Starting from the end of the first day the quantity of bacilli taken up appears to increase gradually until after a roughly 90 to 120 hour stay of the inoculated piece under the frog skin 50 to 70% of the bacilli present were found in the leukocytes. Up to the 10th day this ratio remains roughly constant.

It can be eseablished fairly early that the absolute number of bacilli decreases from day to day to an increasing degree. This decrease becomes noticeable as early as on the 3rd or 4th day. On the 13th or 14th day, because of the very low number of bacilli still present, it is very difficult to estimate the ratio between free bacilli and those taken up by leukocytes. In one such case about 30% were inside the leukocytes. On the 16th day none of the sparse bacilli still present was in the leukocytes.

In general no very marked changes were observed in the bacilli taken up in comparison with those lying free. In the first two days most bacilli still had a normal appearance and stained evenly intensively with methylene blue. After about 42 hours involutional forms were more frequently encountered, characterized in unstained preparations by nodular and knotty swelling and in place by decomposition of the rods; in stained preparations, in addition to the features mentioned above, they were chiefly characterized by the fact that the rods in question did not stain a fine blue color with methylene blue like healthy bacilli, but a dirty, more or less violet tone, which became the paler the more pronounced the degeneration was. These alterations were found both in free bacilli and in those taken up by leukocytes. I did not observe any considerable differences in the speed or intensity of degeneration between the bacilli taken up and those lying free. It is merely a fact that degenerative forms become the more numerous the longer the inoculated piece remains under the frog skin.

As has already been pointed out, the quantity of involutional forms increased from day to day. After 78 hours the bulk of the bacilli had become uncommonly pale, and nodular and lumpy forms were far more numerous than normal forms. Yet even after 7 days some well colored and formed bacilli were still encountered. After 13 days, among the few bacilli still present there were few of normal form, none of normal coloring.

Since the bacilli degenerated to the same degree inside and outside the leukocytes and the quantity of involutional forms increased with time to a growing degree in both cases, one must admit the possibility that those taken up by the leukocytes did not fall victim to the intracellular digestion assumed to exist by Metschnikoff, but, like the bacilli that remained free, died and decomposed as a result of some harmful influence as yet unknown.

Saprophytes, whose involvement in the degeneration of the anthrax bacilli might very well be considered, were apparently blameless of this damage to the virulent bacteria, since in the first experimental series (see table I) they were absent in by far the majority of cases both under the microscope and in the culture plates, and involutional forms occurred just as soon and in such abundance as in those cases in which saprophytes were found beside the anthrax bacilli.

A decrease or a loss of virulence of bacilli subjected to the influence of leukocytes under the frog skin definitely does not take place, as is shown by the short time within which the inoculated mice died. In the first experimental series (table I) the mice died significantly too late, suggesting an apparent weakening, but the cause was merely the mode of inoculation, as small particles of the inoculation piece were placed directly under the skin of the mouse. Here the viable bacilli still present mainly in the inner portion of the piece apparently required some time to grow through to the surface and elicit the infection. When the mode of inoculation was altered (injection of a suspension of the inoculation piece) (table II), most of the mice died at the proper time. In both experimental series the mice inoculated from the first mice (designated with B in the tables), regardless of whether these had died too late or at the proper time, invariably died between the 20th and 23rd hour.

Since recently Lubarsch (Fortschr. d. Med. 1888 No 4) again claimed a weakening of the anthrax bacilli under the frog skin, I believe that particular emphasis should be placed on these results, obtained from numerous experiments.

Colonies grown on gelatin plates were also used for infection experiments on repeated occasions and these also prove that no decrease in virulence took place. One mouse inoculated from a plate prepared from a piece of tissue that had been left under the frog skin for 16 days

and in which only a few colonies had developed, died of anthrax 17 1/2 hours later. According to Lubarsch, on the other hand, cultures from the piece of inoculation tissue are never supposed to kill mice from the 6th day onwards, in other words the bacilli are supposed to be completely exhausted.

The following experiment is cited as being particularly indicative?: a small piece of lung from an anthrax mouse was placed under the dorsal skin of a mouse in the usual manner. After 6 days plate cultures were made from the piece of lung and the gelatinous exudate. Abundant anthrax colonies grew, but mixed with a few saprophytes. Thecolonies developed within the period customary for virulent anthrax to the normal size, so that even a glance at the plates excluded the possibility of any weakening. Three mice were inoculated from the smallest and most stunted colonies, two of which died of anthrax after 18-20 hours and one after about 30 hours. Another mouse inoculated from the last mouse died of anthrax after about 20 hours.

I do not wish to claim that it is impossible that among bacilli that have stayed alive in the piece of inoculation tissue for a prolonged period of time there may here and there be a few weakened ones. But to prove the weakening of individual bacilli in a convincing manner it will be essential to use the plate method and to test the individual anthrax colonies grown (whose weakening can be seen without difficulty if it is pronounced, because of their stunted growth) with animal and culture experiments. By direct use of stab and smear cultures and subsequent cultivation at high temperature, as was done by Lubarsch, an exact demostration of the development of weakening cannot be made, because of the great danger of contamination by rapidly growing saprophytes, as well as because of the more rapid growth and overgrowth of anthrax bacilli that are not weakened.

Metschnikoff's claim that the virulence of the bacilli contained in the inoculated tissue is lost between the 3rd and 5th day has thus been shown to be incorrect. Virulent anthrax bacilli were still present in the inoculation tissue after 16 to 17 days. Naturally the size of the piece of organ placed under the frog skin will have some influence on the longer or shorter life time of the bacilli inside it. Viable bacilli will keep longer in a larger piece than in a small one, since the harmful influences causing the destruction of the bacilli will progress more slowly on the inside of the piece that is larger. I took all the more care to select inoculation pieces that were small and not larger than those used by Metschnikoff. The difference in our results may be due far more to the differences in methods, since the mode of inoculation used by me and the plate cultures give a more reliable indication of the presence of viable bacilli.

Since at the beginning I was of the opinion that possibly the reduced resistance of frogs towards the end of the winter may have influenced the results, I made another series of experiments in May 1887 with strong summer

frogs, the results of which, concordant with the previous ones, are shown in table III.

Experiments with weakened anthrax (that kill mice but not rabbits)made in the manner described above yielded microscopically essentially the same findings as before (see table IV) . Mice inoculated with the inoculation tissue died between 40 and 84 hours later The experiments were continued up to the 7th day.

If the frogs were kept in an incubator at a constant temperature of roughly 23°C, in the first days a strong proliferation of the bacilli contained in the inoculated tissue piece was observed. After 22 hours three frogs had died, probably as a result of an excessively high temperature.

In one of these a few anthrax bacilli could be detected in the liver and heart blood. In the inoculation tissue, there were many long anthrax threads, of which very few were taken up by leukocytes, however. In a live frog examined 24 hours after the inoculation there were 2% bacilli in the leukocytes and about 12% of leukocytes contained bacilli (see table V). The free bacilli in many cases had grown into very long threads traversing several visual fields. The leukocytes had also taken up such long threads, which, in order to find room in the small round body of the cell, were often bent at various angles or wound up in a spiral.

The activity and hunger of the leukocytes appeared to be somewhat increased by the elevated temperature. After 48 hours 27% of the bacilli were already in cells, after 68 hours about 50%, in connection with which it should be noted that here the absolute quantity of bacilli was incomparably greater than in the low temperature experiments.

After 95 hours 31% of the bacilli had been taken up by leukocytes. From this time onwards a distinct decrease in bacilli was observed. In the frogs examined after a period longer than 95 hours increasingly more dead individuals were found. The absolute number of bacilli present was still invariably far greater than in the experiments at low temperature made at the same time, which is readily explained by the fact that in the latter case initial proliferation did not take place. After 9 days most bacilli were involved, whether free or inside leukocytes. The number of degenerated free bacilli was certainly not smaller, but rather greater than that of those taken up by leukocytes. Those consumed by leukocytes could only be recognized in places by colored transverse lines.

Saprophytes were found in large numbers in all cases beside the anthrax bacilli. Some were also contained in the leukocytes.

Aside from the frog mentioned above that died after 23 hours, anthrax bacilli were never found in the organs or in the heart blood of the animals.

The virulence of the bacilli was still intact up to the 7th day; a mouse infected from a piece of inoculated tissue died of anthrax after 25 hours. On the 9th day the bacilli had apparently died, since one inoculated mouse no longer died of anthrax and since no anthrax colonies developed on the plates.

The last mentioned experiments are striking owing to the fact that the anthrax bacilli proliferated amply at the beginning, but finally were nevertheless completely destroyed, indeed more rapidly than in the low temperature experiments. The question arises whether the subsequent inhibition of growth and the dying off of the bacilli can be attributed to the activity of the leukocytes. We observed that the activity of the leukocytes was apparently somewhat increased by the slight elevation in temperature and that they had taken up bacilli in somewhat larger numbers than in the low temperature experiments, but always at the most half the bacilli present were found inside leukocytes. If we wish to assume that this half was impeded in its growth by being enclosed in the cells, then one cannot see why the free bacilli also stopped growing so early and were rapidly destroyed. That the free bacilli were freed only by the preparation, as is objected by Metschnikoff against such findings made by other authors, is impossible. Aside from the fact that the preparation took place very carefully, in the manner given by Metschnikoff, in the case of the free bacilli we were dealing with very large quantities and often with long threads passing through an entire visual field, whose uptake could at best have been achieved by a large number of leukocytes ranged side by side. I can therefore claim with certainty that the final destruction of at least a large part of the bacilli cannot be attributed to their uptake and digestion by leukocytes.

I obtained the following results with frogs kept at a temperature of 25 to 27°C (table VI).

Two frogs were found dead after 23 hours and examined. In the inoculated tissue piece and around it very strong proliferation of bacilli had taken place. Most of these were free; only a few were found inside leukocytes. Not even short anthrax rods were found in the heart blood and organs in great numbers; saprophytes were also few in these sites. A frog killed 24 hours after inoculation gave the same results. Four frogs that had died between 31 and 41 hours after the inoculation yielded similar results. Here again only very rarely could bacilli be observed in the fairly numerous leukocytes that were present.

Whether these frogs had died of anthrax is all the more difficult to decide, in view of the often very low number of bacilli found in the blood and internal organs, since non-inoculated frogs also die fairly rapidly at an elevated temperature.

According to these and the following experiments made at an even higher temperature we cannot exclude the possibility that frogs kept at 30°C and higher are so much reduced in life energy that they can scarcely be regarded as viable animals and that their body, lacking resistance, is permeated by anthrax bacilli in the same way as in dead frogs under whose skin a piece of anthrax-infected organ is placed.

The results obtained with frogs at temperatures between 29 and 37°C ar as follows: Despite all precautions, such as ample and continuous ventilation of the room in which the animals were kept, and ample moisture of the air, it was not possible to keep the animals alive for more than 14 hours. Most died after 5 to 7 hours. In all of them abundant proliferation had taken place around the inoculated tissue piece. In most, anthrax bacilli were also found in the heart blood.

If we consider the collective results of these experiments on frogs it appears at first that Metschnikoff's statement that there is a pronounced accumulation of leukocyte around the anthrax bacilli introduced under the frog skin in pieces or ogan, and that these leukocytes take up large number of anthrax bacilli, was amply confirmed. Similarly a destru ction of the bacilli taken up could definitely be demonstrated inside the leukocytes. My results differ, however, from those of Metschnikoff's in that I observed just as many bacilli, if not more, as those taken up by leukocytes, outsic them in a state of complete degenerative destruction. Furthermore, in my experiments after 16 days under the frog skin viable virulent anthrax bacilli could still be demonstrated in the inoculated tissue piece. We never observed any weakening of the bacilli that were still alive.

The facts that in frogs kept at a considerably elevated temperature the anthrax bacilli proliferate vigorously and even grow into the body of the animal, that at the side of inoculation there is only a small accumulation of leukocytes and very rarely any uptake of bacilli accord with the findings of Metschnikoff obtained in Similar experiments.

The factor which is the most important in evaluating phagocyte activity as a protective arrangement of the organism is apparently the fact that anthrax bacilli under the frog skin are also destroyed in large numbers outside the phagocytes. It is obvious that by establishing this finding Metschnikoff's experiments have suffered considerably in their conclusiveness.

Aside from the observations on Daphnia, the frog experiments are the principal support for Metschnikoff's theory. The experiments on warm blooded animals, performed by Metschnikoff in much smaller numbers, raise more objections to start with and are by no means as conclusive as the frog experiments. Nevertheless it was important to find out to what extent any notable quantities of bacilli were destroyed outside the cells in warm-blooded animals.

II. EXPERIMENTS ON WARM-BLOODED ANIMALS

First of all I repeated Metschnikoff's inoculation experiments with virulent and weakened anthrax on the rabbit ear.

Sterilized, thin-walled glass tubes were filled, in the manner described by Metschnokoff, with a suspension of anthrax pure culture or anthrax-infected organ. These were introduced under antiseptic conditions into a pocket situated under the skin of the ear and after closure of the flesh wound, they were broken off.

A. Experiments with Weakened Anthrax

Experiments were made with such bacilli on four animals. The weakened anthrax originated from a culture which had been kept for 18 days at 42 to 43°C. It killed mice, but not rabbits.

In a first experiment examination of the ear after 22 hours revealed a pronounced accumulation of leukocytes around the inoculation tube. These had taken an abundance of mostly strongly involved bacilli. The free bacilli were also for the most part strongly affected. Upon examination of the culture used for inoculation it was found, incidentally, that this too contained very considerable quantities of involutional forms. After 47 hours only one single very pale bacillus could be found inside a leukocyte. The leukocyte accumulation had progressed almost to pus formation.

In the second and following experiments I experimented with completely fresh cultures free of involutional forms. After 16 hours examination it revealed a fairly significant leukocyte accumulation, although this was not as pronounced as in the previous experiment. The bacilli for the most part lay free, but - a sign of the onset of involution - no longer stained well in most cases.

After 22 hours about 50% of the bacilli were observed to lie inside the leukocytes. Often longish threads were surrounded by several leukocytes. Many of the bacilli taken up were very strongly involved. After 41 1/2 hours

the number of bacilli had become less; some 50% were now inside leukocytes. These, as well as the free ones, mostly gave the impression of distinct involutional forms. After 64 hours there were only very few bacilli present at all, but among them some were still free.

In the other experiments the findings were similar. After about 16 hours an exudate rich in cells was found around the inoculation tube, but most of the bacilli were free and in part in good condition.

After 22 hours some were taken up by leukocytes and these as well as the free ones consisted only of involutional forms. From this point onwards the bacilli gradually disappeared, but so long as any were present, some were always free.

Some experiments made with Pasteur's first anthrax vaccine, gave similar results.

If the glass tubes were filled with completely fresh bouillon culture made from such vaccine, after about 20 hours a serous exudate was observed around them, with a moderate number of leukocytes. The bacilli were almost all free and about half were of normal appearance and had normal staining qualities. After 30 hours the number of leukocytes had increased, while that of the bacilli had decreased. The latter were for the most part degenerated, but at the most half were taken up by leukocytes. After 45 to 48 hours only very sparse, completely degenerated bacilli could be detected between and in the increased number of leukocytes.

If , on the other hand, instead of fresh bouillon culture a suspension of an old culture was used on an oblique agar surface to fill the tubes, so that almost only spores and involutional forms came to be under the skin of the animal, after about 20 hours the accumulation of leukocytes had progressed almost to the point of pus formation. The bacilli were completely degenerated. In contrast to the finding in the experiments with fresh culture, in which around this time most of the bacilli were still free, here more than half of the strongly degenerated bacilli were taken up by leukocytes. After 30 to 45 hours the accumulation of leukocytes was still somewhat further intensifed. Inside the tube and close around it a viscous pus had formed, in which anthrax bacilli could only be found with great difficulty, some free, some taken up, but in either case strongly involved. Foreign microorganisms that may have elicited the purulence were not found either in this or the following experiments.

B Experiments with virulent anthrax

Once again the first experiments were made on three animals with an old

spore culture and a culture rich in involutional forms. Findings were initially similar to those in the experiments with weakened anthrax. The exudate was fairly ample and leukocytes almost as numerous as in the previous experiments. After 17 hours about one third of the bacilli had been taken up by leukocytes. These as well as the free ones were strongly involved. The latter fate of the spores and bacilli at the site of inoculation could unfortunately not be followed in these experiments. The animals all died of anthrax.

In two other experiments suspensions of the spleen of a mouse that had just died of anthrax, in other words very powerful material, was used for inoculation. 20 3/4 hours after inoculation there was a very slight exudate at the site of inoculation and a serous impregnation of the surrounding tissue. Few leukocytes were present in the exudate. None of the latter had taken up bacilli. The bacilli themselves were richly proliferated and all were of normal appearance. The exudate did not increase even later. The animals died of anthrax after about two days.

In the experiments shown in the following table (No. VII), with virulent anthrax on rabbits, somewhat larger quantities of inoculum were used to fill the tubes (0.3 cc on the average). It was found that the local reaction was somewhat more pronounced than in the previous experiments. The tissue in the area surrounding the inoculation tube in all cases revealed severe serous impregnation (anthrax swelling). In the immediate vicinity of the broken tube, when using inoculum rich in involutional forms, there was an ample exudate, which later became almost purulent. In the exudate the bacilli sometimes initially appeared to grow, but later were destroyed, most of them independently from the cell. If bacilli were found in the cells, they were always strongly involved. In the serum that impregnated the tissue around the tube the bacilli proliferated very amply. In this serum there were relatively few leukocytes and these had not taken up any bacilli.

Upon infection with fresh, powerful bacilli the accumulation of leukocytes around the tube was smaller. There was no pus formation. Here too some bacilli degenerated, but most of these were free. At the site of inoculation there soon developed strong proliferation of the bacilli and of these powerful bacilli scarcely a single one was found inside leukocytes. It is not my task here to follow the numerous diversities in local reactions, which are very interesting in themselves. Only very carefully carried out large series of experiments can decide the facts relating to this subject. The relationships between virulent and weakened anthrax on the one hand, and local reactions on the other , are undoubtedly not quite so simple as is assumed by Metschnikoff and Christmas-Dirkinck-Holmfeld. According to my experiments one cannot at least exclude the possibility that the larger or smaller content of involutional forms of the cultures used for injection have some

influence on the intensity of the local reaction; possibly the quantity of bacilli introduced is not a matter of indifference, all of these being points concerning which reliable data can only be furnished after thorough investigations.

What definitely emerges from my experiments, however, and what they are primarily intended to throw light upon is the fact that when bacilli are destroyed in the exudate, this occurs outside the cells in by far the majority of cases.

In experiments on immune animals I was able to confirm the results of Metschnikoff and Christmas-Dirkinck-Holmfeld in so far as the intensity of the local reaction is concerned. Five rabbits were immunized according to the method of Chamberland and Roux, by intravenous injection of 50 cc Bouillon culture from Pasteur's vaccine I against anthrax. About one week after the injection four animals received an injection of about 0.3 cc of a fresh bouillon culture of virulent anthrax under the skin of the ear and one animal the same quantity of Pasteur's vaccine II. After 24 hours all animals developed fairly pronounced reddening of the ear and swelling around the site of inoculation. Upon puncturing the swollen area, a drop of thick pus was evacuated. The microscope revealed the presence of numerous anthrax bacilli in the pus, in some cases in good condition, in others degenerated to a varying degree. By far the greatest number of these bacilli were free. The few found inside leukocytes were not of normal appearance, but proved to be degenerated, although in some cases not to a more pronounced degree than a large part of the free bacilli. After 48 hours all bacilli were very strongly involved. Yet the uptake by leukocytes had scarcely increased.

Since the lumps of pus did not become rapidly reabsorbed, but gradually assumed a more cheesy quality, the presence of bacilli in its contents could be examined even after a fairly prolonged period. By means of Gram's method it was possible to demonstrate the totally degenerated bacilli, but almost all of them free.

III. Microscopic Observations on the Heated Stand

It seems probable on the basis of the experiments described above that the destruction of bacilli in the live body is not solely the task of leukocytes, but that other influences also play a part in their degeneration. However, only individual phases of the reciprocal relationships between bacilli and leukocytes could be observed on the live animal. I hoped to obtain a better insight into some aspects of the problem under consideration by attempting to observe the behavior of leukocytes towards bacilli directly on microscopic

preparations, continuously over a fairly prolonged period of time. There was a possibility that in such experiments one could establish more accurately whether the leukocytes immediately take up the bacilli or whether only after a certain period, and whether virulent and weakened bacilli differ in their behavior in this situation. Such observations also promised more exact information as to whether and to what extend degeneration of bacilli not taken up by leukocytes takes place in animal fluids.

Application of this method seemed all the more indicated since Metschnikoff himself had also used it. He found that upon uniting anthrax bacilli and frog lymph on the heatable stand only the bacilli lying inside leukocytes revealed signs of degeneration and that the leukocytes of animals susceptible to anthrax showed a lesser capacity to take up bacilli in analogous experiments than those of completely or partially immune animals.

My experiments along these lines, which initially were only made to confirm these claims of Metschnikoff's, revealed very striking results, so that it seemed worthwhile to extend them further. I have therefore thoroughly investigated successively the behavior of anthrax bacilli and later a few other bacteria in blood, lymph and several other tissue fluids on various animal species.

EXPERIMENTAL PROCEDURE

For the purpose of microscopic examination drops of the fluid to be examined were placed on a cover glass, inoculated at the edge with a small amount of anthrax bacilli and embedded into a hollow ground slide with paraffin. Such preparations can be observed for a prolonged period and if all precautionary measures are observed, contamination by saprophytes is absolutely impossible for the period of observation.

For inoculation of the drops only fresh, powerful bacilli were used, either in the form of a thin suspension of the spleen of an animal having just died of anthrax or completely fresh anthrax threads distributed in sterilized saline solution, from a roughly 12 hours old bouillon culture.

The flakes of dense anthrax threads floating in such young cultures were fished out with a bent platinum pin and transferred to 10 to 12 cc sodium chloride solution. Even distribution in the saline ran into some difficulties at the beginning; but this can be very satisfactorily achieved if

the fluid is vigorously shaken in a thick-walled test tube with a small amount of sterilized coarse sand or very fine gravel. By this means the threads can be divided into all single bacilli.

The drops of this thin suspension were inoculated at the edge using a small platinum loop, so that about 30 to 100 bacilli reached the drop. The bouillon introduced with the flake when transferring the latter into the saline was so much diluted by the quantity of saline that the very small amounts used for inoculation of the drops undoubtedly did not cause any accompanying transfer of nutrients.

If the observations were to be made at warm blooded temperatures, I used a heating chamber instead of the heatable stand used hitherto, capable of enclosing the entire microscope. This chamber was modified according to the idea described by Sachs; on the whole it resembles an apparatus that is commercially available from Zeiss in Jena, but it has certain advantages over this. The side walls are not made of wood, but are double metal walls. The intermediate space between the walls of the fixed parts, namely the front and posterior walls and the floor, is filled with water, while the side walls that flap outward at the hinges are filled with asbestos. In addition, the entire apparatus save for the copper floor is lined with felt. With this construction it is possible to keep the temperature inside it extremely constant by means of a small gas flame. In order not to have to open an entire side wall, if it should be necessary to move the object, which would result in a considerable reduction of temperature inside the apparatus, an oval opening is made on the left side wall at the level of the microscope stand, which allows insertion of the fingers or the hand and thus movement of the object without appreciable change in temperature of the inside chamber. As a rule this opening is closed by a conical cover. The details of the construction can be seen from the figure.

In a heating chamber of this sort the temperature can easily be kept constant down to fractions of grades for a prolonged period and it has the great advantage over the stand formerly used that the temperature of the object is really that shown by the thermometer. The inner surfaces of the walls are lined during use with several layers of moist blotting paper.

A. Experiments with Frog Lymph and Frog Blood

The plasma rich in leukocytes required for these experiments was obtained in the following manner:

a piece of sterilized cotton was introduced under the dorsal skin of a frog under aseptic conditions. After 24 hours so much lymph had collected around this plug of cotton that by sucking it up with a capillary tube, several drops could be collected, which were then inoculated in the manner described above with anthrax bacilli, and observed for a period of time.

Soon after the beginning of the examination, uptake of bacilli by vigorously moving leukocytes could be observed. Longer threads were as it were encircled by several leukocytes so that formations resembling rose crowns were produced. Processes of degeneration were distinctly visible after a few hours, both in free and in the taken up bacilli, although this was only pronounced and rapid at temperatures between 15 and 18°C.

Two free bacilli, observed continuously at 15°C, showed distinct signas of degeneration after 4 hours. At 18°C involution of individual members was observed in long free threads within a few hours. I also observed definite alterations in several other cases at this temperature in free bacilli within 3 to 6 hours.

The alterations consisted chiefly in the fact that the protoplasm of the bacillus first became granular and the outline became more irregular. Gradually either the granular structure disappeared again, the outlines appeared sharp, but the bacillus itself became paler and disappeared almost completely from view, or else the granulation of the protoplasm increased still further and the bacillus broke up into several pieces. Club-shaped and nodular swellings were also observed fairly often among the dying bacilli. Swelling often amounting to double the normal was also not uncommon. All these alterations could be very clearly observed on free bacilli.

In the case of those lying inside leukocytes, on the other hand, direct observation of the signs of degeneration was somewhat more difficult, since the bacillus, once taken up completely into the protoplasm, cannot be seen very distinctly without the addition of reagents. Only occasionally do we see the alterations of bacilli taken up by leukocytes very clearly in fresh preparations, if these lie bridge-like over a vacuole. After staining of the preparations the signs of decomposition of the other bacilli lying in the cells also become very distinct.

Only a thin, alkaline methylene blue solution was used for staining, which visualizes the alterations in the bacilli from their very beginning to their strongest development in an extremely finely graduated fashion, as described in more detail above. One cannot obtain any finer differences in the degeneration of the bacilli by means of the Metschnikoff's Vesuvin method. At the most it shows whether a bacillus is still viable or whether it is quite dead.

If the preparations kept at 15° to 18°C were stained after about 6 hours, it was found that at least 50% of the free bacilli were degenerated while at the beginning of the experiment in control preparations only normal bacilli were found. The free degenerated bacilli were at least as abundant in all preparations as the ones inside the cells.

At higher temperatures (23 to 24°C) I was not able to observe any change in free bacilli after several hours. Among those in cells, I saw alterations in one case after 18 hours. Whether this result would be constant, however, cannot be determined in view of the small number of experiments.

In the case of the many experiments made between 15 to 18°C growth of the bacilli was never observed, while in an analogous preparation made as control with bouillon, 3 bacilli observed at the same time continuously had increased by 1/10 to 1/3 of their original length, a sign that it was not the low temperature of the preparations that hindered growth.

It has been observed many times that leukocytes take up long threads of bacilli and bend and fold them in the most diversified manner. In some cases vacuoles were seen to form around the bacilli that were taken up, a phenomenon which Metschnikoff regards as symptoms of intracellular digestion of the bacilli by the leukocytes in the manner of ameobae. According to my observations it does not appear to have this significance, since it occurred quite irregularly. Sometimes there were vacuoles in the cells that contained no bacilli at all, in other cases - and this was not so rare - immediately after uptake vacuoles formed around some bacilli, and in the great majority of cells containing bacilli there was no vacuole formation.

Thus from these experiments with frog lymph one cannot deduce that leukocytes play a decisive role in the destruction of bacilli.

Series of observations made analogously in hanging drops of blood taken from the heart of frogs gave practically the same results. From the beginning fairly numerous bacilli were taken up by leukocytes, but always a very large proportion remained free. Free as well as enclosed bacilli proved to be completely degenerated in stained preparations after 5 to 6 hours. In the free bacilli the progressive degeneration could be very clearly observed, even in fresh preparations.

The phenomena were the same in the blood of a toad, save that the regeneration of the bacilli took place somewhat more rapidly (see table IX).

B. Experiments with the blood of warm blooded animals.

In the following experiments with mammalian blood, from the blood issuing from one of the small blood vessels or a small wound a small drop was quickly placed onto a cover glass by means of a platinum loop, inoculated in the manner described above with anthrax bacilli at the edge, after which the cover glass was affixed to a prewarmed hollow ground slide with paraffin.

The preparation was then immediately placed into the heated chamber of the microscope, heated to the blood temperature of the animal in question, and observed.

Naturally care was taken to observe the strictest aseptic conditions when withdrawing the blood from the body and transferring it to the cover glasses. The cover glasses onto which the droplets were brought were sterilized by heating over the flame of a gas burner. Contamination of the blood by foreign microorganisms was therefore very seldom observed.

Since the drops of blood coagulated within a short time on the cover glasses and the bloody serous fluid around the coagulum and pressed out of it collected chiefly at the edge of the drop, the anthrax bacilli were always introduced into the outermost edge of the drop, so that they were surrounded by fluid throughout the duration of the observation and not included in the coagulum. Also, naturally, the greater transparency of the thin peripheral layer made observation considerably easier. As should be noted at this point, coagulation of the blood does not eliminate its bactericide qualities; instead, we learned later from special experiments that defibrinated blood also has these qualities to a considerable degree.

One or two of the preparations made were examined continuously under the microscope. At first the entire preparation was examined to determine whether bacilli had already been taken up by leukocytes, next a few free threads were added and the changes taking place in these were observed. The preparations that were not continuously observed were also stored in the heating chamber of the microscope and after varying periods of time the changes occurring in the bacilli were examined, first in the fresh state and then stained.

On the whole the experiments have revealed that while some of the bacilli are taken up by leukocytes, the greater part remained free and yet degenerated to a varying degree. With regard to the uptake of bacilli by leukocytes and the speed and extend of degeneration of the free bacilli, the blood types of the various animal species revealed fairly considerable differences.

In the next tables (VIII and IX) the behavior of anthrax bacilli is shown in the blood of various animals.

As the time of maximal degeneration I took the time when no further increase could be observed in the alterations taking place in the bacilli in fresh preparations. Taking the mean of a fairly large number of individual observations, it was possible to fix this time point at least approximately. After onset of maximal degeneration the state of bacilli in the preparations still kept at the temperature in question remained stationary for a period, after which growth gradually set in in the drops to which larger quantities of bacilli were added and in which remnats still capable of development were left, so that after a certain period the entire drop was found to be permeated by a dense felt of anthrax threads. In other preparations (for instance exp. 16), there was complete degeneration of all bacilli, and naturally at a later period growth was again arrested.

Of the maximally degenerated preparations a number were always stained with methylene blue and the pictures thus obtained confirmed and supplemented the results obtained with the unstained object.

Bacilli from the same source kept simultaneously at the same temperature in a drop of saline or bouillon, for purposes of control, at the time at which maximal degeneration had taken place in the blood preparation in the first case only showed normal bacilli and in the second considerable growth.

The tables show the results obtained with the various animal species roughly in the succession of the onset of maximal degeneration of the free bacilli. It can be seen that degeneration sets in fastest in man, occurring in one case as early as within 3/4 of an hour and on the average after 1 3/4 hours. However, I should not wish to claim constancy of these differences in blood types from the few experiments performed by me. In one case, after 1/2 hours of constant observation normal bacilli reappeared in the preparations and after 4 hours distinct growth could be observed. Very many bacilli were introduced into the drops and probably only a small fraction of these was damaged.

Many bacilli were taken up by leukocytes, but most of them were free. The ratio between the degeneration of the free bacilli and that of bacilli taken up by the cells is shown in fig. 9, table IV. It is apparent from it to what extend the degenerated free threads exceeded the enclosed ones in number.

Degeneration was almost as rapid in the blood of an immunized sheep as in human blood, being maximal after an hour. After 24 hours degenerative forms were still present, while after 26 hours growth was observed. Fig. 8 shows that the degeneration was not as pronounced as in human blood; it is, however, possible that after an hour maximal degeneration had not yet been reached, as is also suggested by the late onset of growth in these preparations. The number of observations was unfortunately too small to allow any significance to be attached to the time difference. In a non-immune sheep maximal degeneration was only observed after 1 1/2 hours and in these preparations growth started earlier.

In experiments with dog blood at first a test was made with ethe blood of a dog chloroformed until its death. Degeneration in this blood was slight, indeed in most preparations it was almost completely absent and instead of it growth started after a short time. The incomplete nature of the degeneration must perhaps be attributed to the form of death of the animal and the relative lateness of the time of withdrawal of the blood, since dog blood, as is apparent from the two other experiments shown in the table, otherwise has a fairly powerful bactericide effect, which appears to be close to that of humans. Unlike Metschnikoff, I did not observe deterioration of the dog leukocytes, but on the contrary, they appeared to take up a fair number of bacilli.

Bird blood appeared to have a low bacterice effect. Degeneration was slight, in so far as the quantity of degenerated bacilli is concerned. On the other hand the signs of degeneration developed very rapidly. I was also able to observe rapid uptake by leukocytes. It can be seen from fig. 7, however, that the absolute quantity of degenerated free bacilli was very considerable.

The slight quantitative effect appeared to be caused chiefly by the fact that the drop of blood coagulated as it was brought to the cover glass, and aside from a solid coagulum, only very little blood colored fluid remained. Later experiments in which rapid coagulation was avoided by prior defibrination and a larger amount of blood was used left no doubt of the vigorous bactericide effect of bird's blood.

Destruction of bacilli in the blood of rabbits took place more slowly than in the above observations, but nonetheless very completely. On the average the maximum was reached after about five hours and almost all bacilli were found to be involved. After the 28th hour growth was observed here also.

I observed the uptake of bacilli shortly (30 min) after contact of the blood with the bacilli, and upon examination later of the preparations it was found that a considerable proportion of bacilli lay inside cells, even though this was not as large as in the blood of other animals. Thus I am unable to confirm Metschnikoff's claim that rabbit leukocytes on the heatable stand are just as little able to take up virulent anthrax bacilli as in the live animals.

In mouse blood without exception there was hardly any degeneration, but immediate growth. Uptake by leukocytes was very slight.

In addition to blood, other tissue fluids were also included in the investigations, paying particular attention to those containing as few as possible cellular elements, in order to overcome the objection that leukocytes might still be responsible in some way for the degeneration of the bacilli.

The aqueous humor is obviously very suitable from this point of view. As is also admitted by Metschnikoff, it contains very few leukocytes. The pericardiac fluid contains somewhat more leukocytes than the aqueous humor, but still very few.

In spite of this, these three fluids provied to have a considerable bactericide effect in experiments exactly like the previous ones (see table X). Figs. 10 and 11 show the extraordinarily strong degeneration of bacilli in the aqueous humor of a rabbit after about 2 hours. Fig. 10 shows bacilli kept for the same period under the same conditions in a drop of NaCl solution, beside the degenerated bacilli, so as to point out the strong difference between them.

I also investigated roughly how long the blood kept its bactericide effect outside the body. For this purpose I prepared a few blood samples (rabbit blood) in the usual manner, save that before inoculation I left the drops standing for various lengths of time at the animal's body temperature. It was found (see table X) that in drops inoculated after 4-16 hours no degeneration took place, but that instead growth started immediately.

Without for the moment going into any attempt to explain the bactericide properties of animal fluids, we can claim as a definite result of our experiments that the destruction of bacteria is not caused by the activity of leukocytes in these experiments. The experimental results cited and a glance at the figures show this clearly.

I have been able to confirm that some of the bacilli are taken up by leukocytes and degenerated in them, but I also observed that the same processes of decomposition, and to a much greater extend, take place in the large number of free bacilli present, in exactly the same manner. The influence of leukocytes becomes even more improbable if we consider that in fluids very poor in leukocytes (pericardiac fluid and aqueous humor) complete degeneration of the bacilli also took place within a short time. All these observations tend far more to the surmise that the bacilli taken up by the leukocytes were no longer completely normal and that the actual

bactericide factor must be sought in the fluid surrounding the cells. The parallel existing in most experiments between the speed of onset of bacterial destruction and the uptake by leukocytes definitely suggests that such an assumption may be valid. The more rapidly degeneration of the free bacilli takes place, the more we find in the leukocytes. If slow degeneration takes place, the life energy of the leukocytes becomes exhausted and extinguished, after which little uptake into the cells is possible.

In humans and rabbits, where the difference in uptake is the most distinct, it can be seen that the leukocytes do not stay alive longer than 2 1/2 hours, on the other hand, degeneration of bacilli in the rabbit only reaches its maximum after about 3 hours, while in humans it is complete within one hour. Thus the uptake by leukocytes in human blood is far more abundant than in rabbit blood. In mouse blood, in accordance with the very slight effect on free bacilli, only a small number of bacilli were found in leukocytes.

That in the frog, despite the slow degeneration, there was nevertheless considerable uptake is clearly explained by the fact that the leukocytes of cold blooded animals are viable far longer outside the body than those of warm blooded animals. After 5-6 hours I saw frog leukocytes still executing fairly ample movements.

VI. CULTURE EXPERIMENTS

It cannot be seen entirely clearly from the experiments with hanging drops whether the blood only alters the bacilli, or whether the bacilli referred to as degenerated, at least in some cases, actually die, that is to say, can no longer be developed in a good nutritive medium. It seemed all the more desirable to find an answer to this question since Fodor[1], on the basis of his experiments - even though these are burdened with such considerable sources of error that they cannot be regarded as conclusive - has claimed that blood, immediately after its withdrawal from the body, is capable of destroying anthrax bacilli. Fodor has assumed, on the basis of these experiments that the rapid disappearance of microorganisms injected into vessels was caused by the fact that these are killed and destroyed within a short time in the blood.

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1 Deutsche Medizinische Wochenschrift, 1887, No. 34

Prof. Fluegge has therefore urged me to establish, if possible quantitatively, whether and to what extend anthrax bacilli are capable of development in fresh blood taken from the live animal, using culture experiments.

The procedure to be used consisted essentially of adding a small amount of bacilli counted by the plate method to the blood, and after stirring for a period of time, counting it again with the blood. By varying the time periods during which the blood and the bacteria were in contact, or after which the bacteria were added, it would be easy to establish how quickly the blood kills the bacteria and how long it preserves its bactericide properties.

I undertook a large number of experiments of this nature, and found that quite significant quantities of bacilli are rendered incapable of development by the blood.

The experimental procedure was as follows:

Blood issuing from an artery or a vein was caught in a sterilized bottle with a glass stopper in which there was a small amount of very fine sterilized gravel.

The bottle was kept at 38° C until the instant of introduction of the blood, so that no cooling of the blood took place. After the necessary quantity of blood (about 25 to 30 cc) had flown in, the glass stopper was inserted and the bottle vigorously shaken a few times, so that the fine gravel caused a very complete defibrination of the blood.

Defibrination could not be avoided for two reasons. In the first place only thus could the entire quantity of the blood sample act on the bacteria introduced into it. The blood of the animals examined forms a coagulum so quickly after withdrawal, that without prior defibrination at best the bacilli could be mixed with pressed-out serum, not to speak of the difficulty of distributing such rapidly coagulating blood evenly into several vessels. But even if it is possible ot mix the non-defibrinated blood in small portions evenly with the bacilli, later an exact determination of the number of bacilli still contained in it would not be possible, because the clot forming after a short time, which includes a completely underterminable proportion of the bacilli, cannot be even remotely evenly distributed using plate cultures in liquefied gelatin.

It was found in my experiments, however, that upon rapid defibrination of the blood with gravel, its bactericide properties are still quite appreciable.

Samples of 0.5 to 1 cc defibrinated blood (defibrination takes only a few seconds) were immediately introduced by means of a sterilized pipette into small prewarmed test tubes which were closed with cotton plugs, and for protection against evaporation were later also covered with rubber caps.

When using mouse blood, several animals were killed by a blow on the head, the thorax opened under aseptic conditions, the blood drawn with a fine pipette from the opened auricle, brought into test tubes and quickly defibrinated by beating and stirring with a platinum loop. This mode of obtaining blood, as well as the incomplete defibrination when using a platinum loop, often cause failures and the results obtained with mice are therefore not considered sufficiently conclusive.

The blood samples were inoculated immediately after being introduced into the test tubes and placed quickly into an incubator kept at the body temperature of the animal in question. All these manipulations took place so quickly that no appreciable cooling of the blood could take place.

For inoculation of the blood I used an evenly thin spleen suspension of a mouse having just died of anthrax in sterilized NaCl solution. Each time the same small platinum loop was filled from this suspension and added to the blood. It can be seen from the tables that as a rule in one experiment almost the same or at any rate a sufficiently concordant quantity of bacilli was mixed in with the blood (see the figures for the colonies grown in the control plates). There was one objection against a more exact measurement of the fluid containing the bacteria, as for instance with a calibrated capillary tube, namely that too much NaCl solution might be introduced into the blood. In the control plates differences of 50 to 100% are still irrelevant when compared to the enourmous and entirely conclusive results of the experiments.

After inoculation of the blood samples first the number of bacilli introduced was determined. For this the same platinum loop suspension which had been added to the blood was mixed with 8 to 10 cc liquefied edible gelatin and poured onto a plate, taking care that all the gelatin contained in the test tube reached the plate - save the remnants unavoidably remaining on the walls, which, if care was taken, were invariably the same amount.

The control plates prepared in this manner were then kept at a temperature of 22°C and after about 24 hours, by counting the colonies, the quantity of bacilli introduced into the blood was determined.

Of the blood samples placed in the incubator from time to time a few were taken out, and after addition of 8 cc liquefied edible gelatin and

thorough mixing, the viable bacilli still contained in them were counted in exactly the same manner by plate culture. The plates (in some cases Petri dishes) were kept in incubators for 3 to 4 days.

It can clearly be seen from these counts made at varying intervals that blood has the capacity to destroy a fairly considerable number of bacteria. It is also apparent that the various blood types possess this quality to a varying degree (see tables XI to XIV).

The latter differences, however, are not always distinct, because the experiments were quantitatively too different, both as to the quantity of blood and the seeding of bacteria, and therefore they cannot simply be compared with one another. The results obtained on immune and non-immune sheep are to some extent comparable.

In the immune sheep in one case the number of anthrax bacilli dropped from 4578 and 4872 to 185 and 283, and in another case from 11,046 and 9,245 to 427 and 665, while in a non-immune sheep the number had only dropped from 7938 and 8330 to 6664 and 4782. In the second case the decrease was also relatively much less pronounced than in the immune sheep.

Whether this difference between immune and non-immune sheep is constant with regard to the activity of the blood must be established by further experiments; the results obtained by me could have been coincidental, since in the other experiments the number of bacilli destroyed varied so very considerably in the same blood.

In one experiment (see table XI) rabbit blood was able to destroy up to 90,000 bacteria, while in another, out of 7,000 bacteria introduced (see table XI) about 45 to 153 still remained viable. The blood of the non-immune sheep (see table XIII) in the first case also destroyed a few thousand bacilli, while in the second case not even 400 were destroyed. To what these differences are to be attributed cannot be determined without further special series of experiments.

In those cases in which not all the bacilli died off, after a certain period there was proliferation of the remaining bacilli in the blood. In mouse blood the bacilli grew soon after they were introduced.

It is clearly apparent from the tables that after reaching a certain minimum figure there is once again a gradual rise in the quantity of bacilli. The time after which maximal degeneration was reached in the various blood types cannot be exactly determined; much greater series of experiments are required for this. In the case of rabbit blood in one case all the 15,000 bacilli introduced were dead after 1 hour (table XI, exp. 2), while in the other experiments the maximum appears to lie between the 2nd and 3rd hour. In the case of the immune sheep further degeneration of bacilli does not appear to take place after 3 1/2 hours.

An important observation was the fact that the bactericide power of the blood is reduced at all after a certain period, and that the blood then represents a good nutritive medium for bacilli.

It proves that the bactericide effect cannot be attributed to some fixed disinfecting substance in the ordinary sense, for we would then have to assume that this substance would itself become ineffective upon destruction of the bacteria. This assumption, however, is not justified, as is shown by the experiments in which I let the blood stand for a period of time and only then introduced the bacteria. It was found (see table XV) that after standing for 8 hours the bactericide effect of rabbit blood was only very slight and thus was extinguished even without the introduction of bacilli. Therefore the bactericide agent may either be a very volatile or extremely labile substance easily broken down by other components of the blood, or else, which seems more probable, we may be dealing with an enzyme action.

This is suggested for instance by another series of experiments in which I allowed temperatures of 50 to 55°C to act on the blood before adding the bacteria. It was found that dog blood that was heated for 10 and 30 minutes to 52° C had lost its bactericide effect; similarly rabbit blood heated for 45 minutes to 55°C had lost this effect completely (see table XVI). Ten minutes of heating to 48 to 50°, on the other hand, did not entirely eliminate the disinfecting power of the blood.

The temperature at which the blood samples are kept after inoculation, at least so far as rabbit blood is concerned, does not appear to have any appreciable effect between 19 and 38°C on the bactericide action of the blood, as can be seen from table XV.

I also had occasion to make a few preliminary orientating experiments from which I learned the manner in which other animal fluids behave towards anthrax bacilli. We saw above that the aqueous humor and the pericardiac fluid showed the same properties on the heated stand as the blood.

It can be seen from table XVII that the bactericide properties of the pericardiac fluid and the aqueous humor were also confirmed by the culture experiments. Similarly a human pleuritic exudate very poor in cells also revealed very vigorous bactericide properties. In some other experiments with aqueous humor I obtained much less striking results, without being able to determine what the cause of these variations was.

In all the experiments so far we were using only anthrax bacilli ; it seemed to me desirable, however, to consider other bacteria as well, notably saprophytes, in order to see whether they too fall victim to the bactericide effect of the blood.

For these experiments I selected Bacillus megaterium (de Bary and B. subtilis. For inoculation of the blood we used roughly 12 hour old spore-free bouillon cultures.

Bacillus subtilis was invariably completely destroyed after 2 hours (see table XVII). Bacillus megaterium showed a considerable reduction, but only in one case had it completely disappeared.

The blood had no effect at all on Staphylococcus pyogenes.

All these experiments, both with other animal fluids and with other bacteria, are too few in number, however, to allow any general conclusions to be drawn.

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In accordance with the results obtained with direct microscopic observation, it would be observed in all the culture experiments, that animal fluids have a harmful effect on anthrax bacilli and other microorganisms.

In the former experiments I was only able to establish that bacilli suffer morphological degeneration under the influence of blood and other tissue fluids, independently from the leukocyte, but in the last experiments I

was able to prove conclusively that a very large part of the bacilli brought into contact with these fluids is completely killed off, and within a relatively short period of time.

Possibly with an improved method there would be even more complete killing of the bacteria; I consider it at least possible that the strikingly small remnants of bacteria capable of development that occur in some experiments are due to certain experimental errors that are not easy to avoid.

Occasionally the rapid increase in the number of bacilli capable of development in the samples left to stand for more than 6 hours gives the impression that in one part of the bacilli there is not a complete killing, but only a kind of weakening, from which they gradually recover. At other times, on the other hand, we are accustomed to and justified in designating the gacteria exposed to a disinfectant as "dead" if they prove to be incapable of proliferation and the formation of colonies after strong dilution with a good nutritive agent. Clarification both of the small remnants of bacilli still capable of development and the circumstances under which now and again the rapid increase just mentioned in the number of bacilli takes place can only be provided by further series of experiments.

As to the killing of bacteria observed in the culture experiments, we can definitely exclude their uptake by leukocytes as the cause of their destrictuion. Metschnikoff's claim that the destruction of bacilli in the live body came about soley by phagocyte activity must therefore be regarded as inconclusive on the basis of the results of my experiments.

I do not wish for the moment to offer any hypotheses as to the signifiance for the organism of the bactericide property of its fluids, studied here in more detail for the first time, or to what it may be attributed. Only when a larger number of further experiments will have been made will it be possible to arrive at reasonably valid concepts regarding the quality and the quantitative variations of this peculiar bactericide mechanism.

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TABLE 1

Experiment with Frogs and Virulent Anthrax Bacilli at 10-16° C.

I. Experiment number.

II. Inoculated tissue piece examined after.

1. hours

2. days

III. Microscopic findings at the site of inoculation.

1. Fairly many leukocytes. Bacilli for the most part free and almost all of normal appearance.

2-2a . The same . The same.

3. Bacilli partly degenerated, both free ones and those inside leukocytes. 27% bacilli inside leukocytes.

4. Bacilli normal for the most part. No uptake by leukocytes.

5. Involutional forms bery common, but both in free and enclosed bacilli; 28% bacilli in leukocytes.

6. Bacilli for the most part strongly involved. Some 38% bacilli in leukocytes. Many free bacilli strongly degenerated.

7. Bacilli very strongly degenerated

8. Few bacilli. None found in leukocytes. Many saprophytes.

9. Bacilli very strongly degenerated, both free ones and those in leukocytes. 63% bacilli in leukocytes.

10. Free and enclosed bacilli strongly degenerated. Few bacilli.

11. Few bacilli. Almost all strongly involved. 38% in leukocytes

12. All bacilli degenerated, some in leukocytes.

13. Very few bacilli. All involved. Some in leukocytes.

14. Very few bacilli. All strongly involved, some in leukocytes.

IV. Results of plate cultures.

1. A few saprophytes.

2. Only anthrax colonies.

3. Almost only saprophytes.

4. Only anthrax colonies.

5. A few saprophytes, few anthrax colonies.

6. No anthrax colonies.

V. Mice inoculated from the inoculated tissue piece died after:

1. hours.

2. after

3. day.

Tabelle I.

Versuche mit Präparaten und virulenten Milzbrandbacillen bei 19 bis 18° C.

I. Vers.-Nr.	II. Das Impfstück wird untersucht nach:	III. Mikroskopischer Befund an der Impfstelle	IV. Ergebnis der Plattenculturen	V. Die vom Impfstück geimpften Mäuse sterben nach:
5	15 Stunden	Ziemlich viele Leukocyten. Bacillen sehr, allergrößten Theil frei und fast sämmtlich von normalem Aussehen.	Einige Saprophyten.	1 2
6	15 „	dergl.		36 Std. B nach 39 Std.
7	16 „	dergl.	Nur Milzbrandcolonien.	36 „ B „ 12 „
21	22 „	Bacillen theilweise degenerirt, sowohl freie wie in Leukocyten liegende. 27 Procent der Bacillen in Leukocyten.	„	96 „
17	40 „	Bacillen meistens normal. Keine Aufnahme durch Leukocyten.	„	40 „
15	42 „	Involutionsformen sehr häufig. Doch gleichmäßig bei freien und aufgenommenen Bacillen; 29 Procent Bacillen in Leukocyten.	„	36 „
19	47 „	Bacillen meistens stark involvirt. Etwa 60 Procent der Bacillen in Leukocyten. Viele freie Bacillen stark degenerirt.	„	21 „
20	72 „	Bacillen sehr stark degenerirt; 31 Procent in Leukocyten.	„	39 „ B „ 20 „
8	90 „	Wenige Bacillen. In Leukocyten konnten keine gefunden werden. Viele Saprophyten.	Fast nur Saprophyten.	40 „
23	91½ „	Bacillen sehr stark degenerirt, sowohl frei, wie in Leukocyten. 63 Procent Bacillen in Leukocyten.	Nur Milzbrandcolonien.	30—60 Std.
13	100 „	Freie und in Leukocyten liegende Bacillen stark degenerirt. Wenige Bacillen.	„	
24	114½ „	Wenige Bacillen. Fast alle stark involvirt. 30 Proc. in Leukocyten.	„	46 Std.
14	7 Tagen	Alle Bacillen degenerirt, z. Th. in Leukocyten.	„	7 Tag. B nach 23 Std.
18	10 „	Sehr wenige Bacillen. Alle involvirt. z. Th. in Leukocyten.	Einige Saprophyten, wenige Milzbrandcolon.	60 Std. B „ 28 „
16	13 „	Sehr wenige Bacillen. Alle stark involvirt. z. Th. in Leukocyten.	Keine Milzbrandcolonien.	60 „ B „ 28 „

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TABLE 2

Experiments with Frogs. Temperature 10 - 16° C.

I. Experiment number.

II. The inoculated tissue piece was examined after:

1. hours.

2. days.

III. Microscopic findings.

1. Fairly many leukocytes. Few involved bacilli. 1% bacilli in leukocytes.

2. Involutional forms more common. Most free. 41% of bacilli in leukocytes.

3. Most bacilli pale in color. 80% bacilli in leukocytes.

4. Fewer bacilli. Many involved, free. 57 1/2 % bacilli in leukocytes.

5. Same; 71% bacilli in leukocytes.

6. Few bacilli. Most involved. 65% bacilli in leukocytes.

7. Few bacilli. Hardly any normal. 70% bacilli in leukocytes.

8. No normal bacilli. 30% bacilli in leukocytes.

9. Several long threads strongly involved and free. In some leukocytes strongly degenerated bacilli.

10. Free degenerated bacilli, but very sparse . No bacilli found in leukocytes.

IV. Results of plate culture.

1. No anthrax colonies.

2. A few anthrax colonies.

3. A few anthrax colonies and saprophytes.

V. Mice inoculated from the inoculated tissue piece died after:

1. 23 hours

2. Did not die

3. Did not die. Inoculated from a colony died of anthrax after 17 1/2 hours.

Tabelle II.

Versuche mit Präparaten. Temperatur 10 bis 18° C.

No.	Das Impfstück wurde untersucht nach:	Mikroskopischer Befund	Resultate der Plattencultur	Die vom Impfstück geimpften Mäuse starben nach:
36	20½ Stunden	Ziemlich viel Leukocyten. Wenig involvirte Bacillen. 1 Procent Bacillen in Leukocyten.		23 Stunden
38	47½ „	Involutionsformen häufiger. Zum grossen Theile frei. 41 Procent der Bacillen in Leukocyten.		24½ „
37	70½ „	Die meisten Bacillen blass gefärbt. 80 Procent Bacillen in Leukocyten.		28 „
39	96½ „	Weniger Bacillen. Häufig involvirte, freiliegend. 51½ Procent Bacillen in Leukocyten.		
39	120 „	Desgleichen; 71 Procent Bacillen in Leukocyten.		30—36 „
40	7 Tagen	Wenig Bacillen. Meist involvirt. 68 Procent Bacillen in Leukocyten.		25½ „
41	10 „	Wenig Bacillen. Kaum noch normale. 70 Procent Bacillen in Leukocyten.		30—40 „
42	14 „	Kein normaler Bacillus. 80 Procent Bacillen in Leukocyten.	Keine Milzbrandcolonieen viel Saprophyten.	Nicht gestorben
43	16 „	Mehrere lange Fäden stark involvirt und frei. In einigen Leukocyten stark degenerirte Bacillen.	Einige Milzbrandcolon.	23½ Stunden
44	17 „	Freie degenerirte Bacillen, doch sehr selten. In Leukocyten waren keine Bacillen zu finden.	Einige Milzbrandcolon. und Saprophyten.	Nicht gest. Von einer Colonie geimpft todt nach 17½ St. an Milzbr.

TABLE 3

Experiments with summer frogs and virulent anthrax bacilli at 17-22° C.

I. Experiment number .

II. The inoculated tissue piece examined after:

1. day

2. days

III. Microscopic findings at the site of inoculation.

1. Slight uptake by leukocytes.

2. Gradual decrease of bacilli. Degeneration the same in free and enclosed bacilli.

3. Almost all bacilli involved.

IV. Results of plate culture.

1. Only anthrax colonies.

2. Same.

V. Mice inoculated from the inoculated tissue piece died after:

1. All mice died at the usual time (20 to 24 hours) of anthrax.

Tabelle III.

Versuche mit Hammerfröschen u. virulenten Milzbrandbacillen bei 17—22° C.

I. Vers.-Nr.	II. Das Impfstück wird untersucht nach:	III. Mikroskopischer Befund an der Impfstelle	IV. Ergebnis der Plattencultur	V. Die vom Impfstück geimpften Mäuse sterben nach
95	1 Tag	1 Geringe Aufnahme durch Leukocyten		1 Alle Mäuse starben zur gewöhnlichen Zeit (20 bis 24 Stunden) an Milzbrand.
96	4 Tagen	2 Allmähliche Abnahme der Bacillen. Degeneration in gleichem Masse an den freien und aufgenommenen Bacillen	1 Nur Milzbrandcolon.	
97	6 „			
98	7 „			
99	8 „			
100	14 „	3 Bacillen fast sämmtlich involvirt	2 Nur Milzbrandcolon.	

TABLE 4

Experiments with frogs and anthrax bacilli weakened for 18 days at 10 to 16° C.

I. Experiment number..

II. The inoculated tissue piece is examined after:

1. hours.

2. days.

III. Microscopic findings at the site of inoculation.

1. Bacilli free. Most in good condition.

2. Most bacilli involved. A large part inside leukocytes.

3. Bacilli partly free, partly inside leukocytes.

4. Most bacilli involved .

5. The same

IV. Results of plate culture.

1. Only anthrax colonies.

2. Almost only anthrax colonies.

V. Mice inoculated from the piece of inoculated tissue died after:

1. hours

2. days.

Tabelle IV.

Versuche mit Fröschen und 18 Tage abgeschwächten Milzbrandbacillen bei 10 bis 16° C.

I	II	III	IV	V
Versuchs-Nr.	Das Impfstück wird untersucht nach:	Mikroskopischer Befund an der Impfstelle	Ergebnisse der Plattencultur	Die vom Impfstück geimpften Mäuse sterben nach:
90	1 26 Stunden	1 Bacillen frei. Meist gut erhalten.	1 Nur Milzbrandcolon.	
90	47 „	2 Bacillen grösstentheils involvirt. Ein grosser Theil in Leukocyten.	2 Fast nur Milzbrandcolonien.	1 66 Stunden
90	50 „	3 Bacillen theils frei, theils in Leukocyten.		
91	64 „	4 Bacillen meistens involvirt. u. Th. in Leukocyten.	Fast nur Milzbrandcolonien.	60 „
92	91 „	5 dergl.		84 „
93	2 5 Tagen	dergl.	Fast nur Milzbrandcolonien.	40—46 Std.
94	7 „	dergl.		2 4 Tagen

TABLE 5

Experiments with fresh and virulent anthrax bacilli at 23°C

I. Experiment number .

II. The piece of inoculated tissue was examined after:

1. hours.

2. days.

III. Microscopic findings at the site of inoculation:

1. (Frog dead). The bacilli had grown into long threads at the site of inoculation. No anthrax bacilli in the heart blood or liver.

2. (Frog dead). Saprophytes at the site of inoculation. Anthrax bacilli had grown into long threads of normal appearance. Rare uptake by leukocytes.

3. (Frog dead). As before. A few short anthrax bacilli in the heart blood and liver.

4. (Frog alive). Anthrax bacilli grown into long threads. Few saprophytes. 2% bacilli in leukocytes.

5. (Frog alive). About 27% of bacilli in leukocytes.

6. (Frog dead). Fairly many saprophytes. About 50% anthrax bacilli in leukocytes.

7. (Frog dead). About 20% anthrax bacilli in leukocytes.

8. (Frog alive). About 31% bacilli in leukocytes. A large part of bacilli stains poorly. But there are just as many free as enclosed degenerated bacilli.

9. (Frog dead). About 12% bacilli in leukocytes. Number of bacilli considerably lower than before.

10. 48% of bacilli in leukocytes. Number of bacilli very low. Most very pale and involved, both enclosed and free.

IV. Result of plate culture

1. No anthrax colonies.

2. Same.

V. The mice inoculated from the piece of inoculated tissue died after:

1. Did not die of anthrax.

Tabelle V.

Versuche mit Präparaten und virulenten Milzbrandbacillen bei 38° C.

Vers.-No.	Das Impfobject wird untersucht nach:	Mikroskopischer Befund an der Impfstelle	Impfresultat der Platten-culturen	Die zum Impfobject gehörigen Mäuse starben nach:
26	23 Stunden	(Frosch todt). Die Bacillen an der Impfstelle zu langen Fäden ausgewachsen. In Herzblut u. Leber keine Milzbrandbacillen.	—	—
27	23 „	(Frosch todt). Saprophyten an der Impfstelle. Milzbrandbacillen zu langen Fäden ausgewachsen, von normalen Aussehen. Selten Aufnahme durch Leukocyten.	—	—
28	23 „	(Frosch todt). Wie vorher. In Herzblut und Leber einige kurze Milzbrandbacillen.	—	—
25	24 „	(Frosch lebendig). Milzbrandbacillen zu langen Fäden ausgewachsen. Wenig Saprophyten. 2 Proc. der Bac. in Leukocyten.	—	84½ Std.
29	48 „	(Frosch lebendig). Etwa 27 Procent der Bacillen in Leukocyten.	—	27 „
32	60 „	(Frosch todt). Ziemlich viel Saprophyten. Etwa 50 Proc. Milzbrandbac. in Leukocyten.	—	—
34	60 „	(Frosch todt). Etwa 20 Proc. Milzbrandbacillen in Leukocyten.	—	—
30	96 „	(Frosch lebendig). ca. 81 Proc. Bacillen in Leukocyten. Ein grosser Theil der Bacillen färbt sich schlecht. Doch sind ebensoviele der degenerirten frei, wie aufgenommen.	—	37 Std.
33	7 Tagen	(Frosch todt). Etwa 12 Proc. Bacillen in Leukocyten. Zahl der Bacillen bedeutend geringer wie vorher.	Keine Milzbrandcolonien.	26 „
31	9 „	40 Procent der Bacillen in Leukocyten. Zahl der Bacillen sehr gering. Die meisten sehr blass und involvirt und zwar ebensowohl die aufgenommenen wie die freien.	2 dergl.	stirbt nicht an Milzbrand.

TABLE 6

Experiments with frogs and virulent anthrax bacilli at 25 to 30° C.

I. Experiment number :

II. Time of examination of the piece of inoculated tissue.

III. Microscopic findings.

1. (Frogs dead) . The bacilli grown into long threads. Rare uptake. Short anthrax rods in heart blood and liver.

2. (Frog alive). Ample growth of bacilli at the site of inoculation. Few leukocytes. Very rare uptake. Anthrax bacilli in the heart blood and liver.

3. (Frogs dead) (not before the 31st hour) . Bacilli strongly proliferated. Fairly many bacilli in heart blood and liver.

4. Same.

5. (Frog alive) . Strong proliferation of bacilli. Leukocytes fairly common, but very seldom containing bacilli. Anthrax bacilli in heart blood.

Tabelle VI.

Versuche mit Fröschen und virulenten Milzbrandbacillen bei 25—30° C.

Versuch	Zeit der Untersuchung des Impfstücks	III. Mikroskopischer Befund
51	23 Stunden	(Frösche todt). Die Bacillen sind zu langen Fäden ausgewachsen. Selten Aufnahme. In Herzblut u. Leber kurze Milzbrandstäbchen.
52	23 "	
77	24 "	2 (Frosch lebendig). Üppiges Wachsthum der Bacillen an der Impfstelle. Wenige Leukocyten. Sehr selten Aufnahme. In Herzblut und Leber ebenfalls Milzbrandbacillen.
78	41 "	3 (Frösche todt) [nicht vor der 31. Stunde]. Bacillen stark gewuchert. Keine Aufnahme durch Leukocyten. Ziemlich viele Bacillen in Herzblut und in der Leber.
80	41 "	
82	41 "	4 desgl.
83	41 "	
79	48 "	5 (Frosch lebendig). Bacillen stark gewuchert. Leukocyten nicht selten, doch sehr selten Bacillen enthaltend. Milzbrandbacillen im Herzblut.

(10)

TABLE 7

Experiments with virulent anthrax bacilli in the rabbit.

I. Nature of material used for inoculation.

1. 6 week old gelatin culture, which in control preparations revealed almost only spores and involutional forms.

2. Spleen suspension made from a mouse having died of anthrax. 9 hours after death of animal. (many bacilli already degenerated).

3. Eight day old bouillon culture with mostly normal bacilli.

4. Same. Injection of 0.3 cc under the skin of the ear.

5. Six week old gelatin culture, containing al most exclusively spores and involutional forms

Tabelle VII.

Versuche mit virulenten Milzbrandbacillen am Kaninchen.

I Art des zur Impfung verwandt. Materials	II Befund nach 20 Stunden	III Befund nach 30 Stunden	IV Befund nach 45 Stunden	V
1 6 Wochen alte Gelatinecultur, welche in Controlpräparaten fast nur Sporen und Involutionsformen zeigt.	Ziemlich starke Schwellung um das Impfröhrchen. In dessen nächster Umgebung viele Leukocyten. Bacillen sehr reichlich, zum grossen Theile gut gefärbt (geronnen). Doch auch viele involvirte. Von letzterem ein kleiner Theil in Leukocyten.	Im Röhrchen und in dessen nächster Umgebung viele Bacillen. Etwa die Hälfte involvirt. Aufnahme nicht häufig.	Im Eiter massig viele Bacillen, fast sämmtlich degenerirt, doch meistens frei. In der ganzen Ausdehnung des Einschnitt in der Nähe des Röhrchens von dem Gewebe quellenden Flüssigkeit reichliche normale Bacillen; wenig Leukocyten.	50
2 Aufschwemmung von Milz einer an Milzbrand gestorbenen Maus. 9 Stunden nach dem Tode des Thieres. (Viele Bacillen schon degenerirt)	Schwellung um das Impfröhrchen. In sodat in der nächsten Umgebung desselben ziemlich viel Leukocyten. Bacillen reichlich. Zum grössten Theil degenerirte wie degenérirt. Ein kleiner Theil der letzteren in Leukocyten.	Im zellarmen Exsudat in der nächsten Röhrchens ziemlich viel Bacillen. Mehr, der Aufnahme durch Leukocyten.	Im Eiter um das Röhrchen wenige ganz degenerirte Bacillen. Dieselben Im Serum viele gut gefärbte Bacillen. wenig Leukocyten. Keine Aufnahme.	2
3 9 Tage alte Bouilloncultur mit zum grossen Theil normalen Bacillen	Exsudat um das Röhrchen ziemlich reichlich. Ziemlich viele Leukocyten. Bacillen sehr reichlich, fast alle normal. Einige degenerirte frei. Sehr selten Aufnahme.	Keine Eiterbildung Im übrigen wie vorher.	In der das Gewebe in der Umgebung des Impfröhrchens durchtränkenden Flüssigkeit ziemlich reichliche Leukocyten, ungemein viele normale Bacillen. Einige degenerirte frei und in Leukocyten.	3
4 dergl. Injection von 0.3 cc unter die Haut des Ohres.	Etwas weniger Leukocyten, sonst wie vorher.	4 Wie oben.	4 Wie oben.	4
5 6 Wochen alte Gelatinecultur, fast nur Sporen und Involutionsformen enthaltend.	Leukocytenreiches Exsudat. In demselben nur degenerirte Bacillen. Ein kleiner Theil derselben ist von Leukocyten aufgenommen.	Im Serum aus dem Gewebe, in der Umgebung des Röhrchens ziemlich viele, theils massig viele, sämmtlich normale Bacillen. Wenig Leukocyten. Keine Aufnahme.		5
6 Aufschwemmung von 18 Stunden alter Bouillon-cultur in NaCl-Lösung. Nur normale Bacillen.	Ziemlich starke seröse Durchtränkung des Gewebes um das Impfröhrchen. Im letzterem wenig Leukocyten. Bacillen fast alle normal. Einige degenerirte frei. Ein solcher in einem Leukocyten.	Keine Zunahme der Leukocyten. Reichlich Bacillen. Die selben fast sämmtlich normal; keine Aufnahme.		48 Stunden an Milzbrand

llon culture in NaCl solution.

ound the inoculation tube. In its
cytes. Very abundant bacilli, for
liferated), but many involved.
in leukocytes.

tube. Fairly many bacilli in
he most part degenerated. Some

abundant. Fairly many leukocytes.
all normal. Some free degenerated

herwise as above.

ly degenerated bacilli in exudate.
ytes.

egnation of the tissue around the
rtes in serum. Almost all bacilli
ed. One degenerated bacillus in

III. Finding after 30 hours.

1. In the tube and its immediate vicinity formation of pus. Not many bacilli in pus. About half involved. Not many enclosed.

2. Fairly many bacilli in the cell-rich exudate in the immediate vicinity of the tube. More degenerated that before. Rare uptake by leukocytes.

3. No pus formation. Otherwise as before.

4. As above

5. Moderately numerous , all normal bacilli in the serum from the tissue in the vicinity of the tube. Few leukocytes. No uptake.

IV. Finding after 45 hours.

1. Moderate number of bacilli in pus, almost all degenerated, but mostly free. In the fluid issuing from the tissue from a cut close to the tube abundance of normal bacilli; few leukocytes.

2. Few completely degenerated bacilli in the pus around the tube. These mostly free. Many well stained bacilli in the serum, few leukocytes. No uptake.

3. In the fluid impregnating the tissue in the vicinity of the tube fairly numerous leukocytes , unusually many normal bacilli. Some degenerated ones free or in luekocytes.

4. As above.

V. Animal died after :

1. 50 hours, of anthrac

2. 52 hours, of anthrac

3. about 50 hours, of anthrax.

4. 48 hours, of anthrac

5. 48 hours, of anthrac.

TABLE 8

Experiments with the blood of various animal species and anthrax bacilli using the hanging drop.

I. Experiment number :

II. Temperature of the drop in degrees C.

III. Animal species.

1. human
2. dog.
3. chicken

Tabelle VIII.

Versuche mit Blut verschiedener Thierarten und Milzbrandbacillen im hängenden Tropfen.

Vers.-Nr.	Temperatur d. Tropf. Grad C.	Thier-species	Zahl der Versuche	Zeit der maximalen Degeneration nach:	Von Leukocyten aufgenommen	Bemerkungen
1	37·5	Mensch	4	1 Stunde	Viele Bacillen	1 81 Bacillen aufgenommen. 36 Bacillen degenerirt und frei.
2	37·5	"	4	¾ "	"	2 In nach 1¾ Stunden gefärbten Präparaten nur noch wenige normale Bacillen.
3	37·5	"	2	1 "	"	3 In gefärbten Präparaten nur noch sehr wenige normale Bacillen, viele degenerirte frei.
4	37·5	"	1	1¾ "	"	4 Im nach zwei Stunden gefärbten Präparat nur involvirte Bacillen, frei und aufgenommen.
5	37·5	"	2	1 "	"	5 Im gefärbten Präparat fand sich nur noch ein normaler Bacillus.
6	37·5	"	1	1¾ "	"	6 Sehr viele freie Bacillen stark degenerirt.
7	37	2 Hund	6	2¾ "	2 Mässig viele Bacillen	7 (Thier zu Tode chloroformirt). Wenig degenerirte Bacillen, davon 50 Procent frei. Meistens nach kurzer Zeit Wachsthum.
8	37	"	6	1 "	"	8 Nach 4 Stunden noch Degenerationsformen. Nach 24 Stunden starkes Wachsthum.
9	37	"	4	1¾ "	"	
10	40—41	3 Huhn	6	2¾ "	Viele Bacillen	9 Nur in der Mitte des Tropfen Degeneration.
11	40—41	"	6	1¾ "	"	10 Nach 24 Std. starkes Wachsthum.
12	40—41	4 Taube	6	1¾ "	"	1 Wenig Degenerationsformen. Nach 3¼ Stunden Wachsthum.
13	37·5	5 Immuner Hammel	6	1 "	Zieml. viele Bacillen	2 Nach 24 Stunden noch kein Wachsthum.
14	37·5	6 Hammel	6	1¾ "	"	3 Nach 24 Std. üppiges Wachsthum.

Im Ganzen ergaben diese Versuche, dass zwar ein Theil der Bacillen von den Leukocyten aufgenommen wurde, dass aber der grössere Theil derselben frei blieb und dennoch mehr weniger stark degenerirte. Betreffs der Aufnahme der Bacillen durch Leukocyten und der Schnelligkeit und Ausgiebigkeit der Degeneration der freien Individuen zeigten die Blutarten der verschiedenen Thierspecies ziemlich bedeutende Differenzen.

on after :

acilli.

bacilli degenerated and free.

after 1 3/4 hours only few normal bacilli

very few normal bacilli, many free degene-

ter two hours only involved bacilli, free and

5. In stained preparation only one normal bacillus found.

6. Very many free bacilli severely degenerated .

7. (Animal chloroformed until dead). Few degenerated bacilli, of these 50% free. As a rule growth starts within a short time.

8. Degenerated forms still present after 4 hours. Pronounced growth after 24 hours.

9. Degeneration only in the middle of the drop.

10. Pronounced growth after 24 hours.

11. Few degerative forms. Growth after 2 1/2 hours .

12. No growth yet after 24 hours.

13. Ample growth after 24 hours.

Table 9 (continuation of table 8, title the same, heading same. Only those requiring translation repeated).

III. Animal species.

1. Rabbit .

2. Mouse

3. Frog

4. Toad

V. Time of maximal degeneratio

1. 2 1/4 hours.

2. about 5 hours.

3. No degeneration.

4. 5 hours.

Tabelle IX.

Versuche mit Blut verschiedener Thierarten und Milzbrandbacillen im hängenden Tropfen

Temperatur d. Tropf. Grad C.	Thierspecies	Zahl der Versuche	Zeit der maximalen Degeneration nach	Von Leukocyten aufgenommen	Bemerkungen
37	Kaninchen	2	2¼ Stund.	Mässig viele Bacillen	
37	"	1	6 "	"	Die ersten Bacillen degenerirten nach ¾ Stunden. Degenerirte Bacillen meist frei. Nach 24 St. kein Wachsth., nur Degenerationsform.
37	"	6	5¼ "	"	Alle Bacillen degenerirt, meist frei. Nach 23 Std. Wachsthum.
37	"	2	ca. 5 "	"	Anfang der Degeneration nach 45 Minuten.
37	"	4	—		In zwei nach 28 Stunden untersuchten Präparaten Beginn des Wachsthums neben Degenerationsformen. In zwei Präparaten nur Degenerationsformen.
37	"	1	—		Im nach 23 Std. untersuchten Präparate fast nur Degenerationsform. Nach 47 Std. üppiges Wachsthum.
37	Maus	2	Keine Degeneration	Wenige Bacillen	Nach 1½ Std. hat schon Wachsthum stattgefunden; nach 3 Std. ist dasselbe sehr deutlich.
37	"	4	"	"	Angl.
37	"	4	"	"	
17–19	Frosch	1	5 Stund	Viele Bacillen	Gleichmässige Degeneration der freien u. aufgenommenen Bacillen.
16–17	"	5	5 "	"	
16–17	"	5	ca. 6 "	"	
16–17	Kröte	5	7¾ "	"	

VI. Taken up by leukocytes.

1. Moderately many bacilli.

2. Few bacilli

3. Many bacilli

VII. Remarks.

1. The first bacilli degenerated after 3/4 hour. Degenerated bacilli mostly free. After 24 hours no growth, only degenerative forms.

2. All bacilli degenerated, mostly free. A growth after 23 hours.

3. Onset of degeneration within 45 minutes.

4. In two preparation examined after 28 hours, onset of growth in conjunction with degenerative forms. In two preparations only degeneration.

5. In preparations examined after 23 hours almost solely degenerative forms. After 47 hours, ample growth.

6. Growth has already taken place after 1 1/2 hours; growth very distinct after 3 hours.

7. Same.

8. Same degeneration of free and enclosed bacilli.

TABLE 10

Experiments with anthrax bacilli and some animal fluids in hanging drops.

I. Experiment number -

II. Temperature of the drop in ^{o}C

III. Animal speicies.

1. Rabbit

IV . Nature of the fluid.

1. Aqueous humor.

2. Pericardiac fluid.

V. Number of experiments.

VI. Time of macimal degeneration after -

VII. Remarks .

1. Only severely degenerated forms to be found in stained preparation.

2. In unstained preparation the bacilli no longer visible after 1 hour.

3. Same.

TABLE 10 a

Experiments with subsequent inoculation of blood samples.

(Headings as in 10, except for VI).

III. Animal species.

1. Rabbit.

IV. Nature of the fluid.

1. blood

VI. Inoculation of samples after.

VII. Remarks.

1. Few degenerated bacilli. Growth within a short time.

2. No degeneration . Ample growth within a short time.

3. Rapid Growth. No degeneration .

4. Same.

Tabelle X.

Versuche mit Milzbrandbacillen und einigen thierischen Flüssigkeiten im hängenden Tropfen.

Vers.-Nr.	Temperatur d. Tropf. Grad C.	Thierspecies	Art der Flüssigkeit	Zahl der Versuche	Zeit der maximalen Degeneration nach	Bemerkungen
29	38	Kaninchen	Humor aqueus	2	1 Stunde	Im gefärbten Präparat sind nur noch hochgradigste Degenerationsformen zu finden.
30	38	"	"	4	1 "	Nach 1 Std. sind im ungefärbten Präparat die Bacillen nicht mehr wahrzunehmen.
31	38	"	Liquor pericardii	2	1 "	dergl.

Table 10 a. Versuche mit nachträglicher Impfung der Blutproben.

Vers.-Nr.	Temperatur Grad C.	Thierspecies	Art der Flüssigkeit	Zahl der Versuche	Impfung der Proben nach	Bemerkungen
33	37	Kaninchen	Blut	4	2½ Std.	Wenige Bacillen degenerirt. Nach kurzer Zeit Wachsthum.
34	37	"	"	5	16 "	Keine Degeneration. Nach kurzer Zeit üppiges Wachsthum.
35	37	"	"	5	22 "	Rasches Wachsthum. Keine Degeneration
36	37	"	"	5	28 "	dergl.

Tabelle XI.

Versuche mit Milzbrandbacillen und Kaninchenblut.

Vers.-No.	Temperatur, bei welcher die Proben nach der Impfung standen	Zeitdauer zwischen Impfung und Anfertigung der Platten	Zahl der gewachsenen Colonien
1	37—38° C.	1 Contr. sofort	94000—90000—27000
2	"	2 nach 1 Std.	38570—70
"	"	4 " 4 "	0
"	"	1 " 5 "	0
3	"	4 Contr. sofort	16015—19707—16006—12510
"	"	2 nach 1 Std.	0—5
"	"	2 " 2 "	2—8
4	"	2 Contr. "	7000—6930
"	"	2 nach 3 Std.	45—158
"	"	1 " 4 "	977
"	"	1 " 5 "	461
"	"	1 " 22 "	1668
5	"	2 Contr.	22—9
"	"	2 nach 2 Std.	0—0
6	"	2 Contr.	9—11
"	"	2 nach 2½ Std.	0—0
7	"	2 Contr.	952—2060
"	"	2 nach 2 Std.	25—1
8	19—21°	2 Contr.	7000—6930
"	"	1 nach 4 Std.	9
"	"	1 " 6 "	23
"	"	1 " 22 "	0

Tabelle XII.

Versuche mit Milzbrandbrandbacillen und Blut von Mäusen.

9	38° C.	2 Contr. sofort	Unzählig
"	"	2 nach 2 Std.	"
"	"	1 " 3 "	"
"	"	2 " 3½ "	"
10	36—38° C.	2 Contr. sofort	952—986
"	"	1 nach 1 Std.	665
"	"	1 " 2 "	339
"	"	1 " 3 "	708
"	"	1 " 4 "	2499
"	"	1 " 5 "	728

2. Versuch mit Milzbrandbacillen und Taubenblut.

11	41° C.	2 Contr. sofort	24—40
"	"	2 nach 2½ St.	0—0

Tabelle XIII.

Versuche mit Milzbrandbacillen und Blut eines nicht immunen Hammels.

Vers.-No.	Temperatur, bei welcher die Proben nach der Impfung standen	Zeitdauer zwischen Impfung und Anfertigung der Platten	Zahl der gewachsenen Colonien
12	37·5° C.	2 Contr. sofort	7938—8830
"	"	2 nach 1 Std.	4268—4312
"	"	2 " 2 "	2499—7321
"	"	2 " 3 "	6664—4708
"	"	2 " 4 "	11025—8136
"	"	2 " 5 "	Unzählbar
"	"	2 " 6 "	"
"	"	2 " 21 "	"

3. Versuche mit Blut eines Hammels, der 48 Stunden nach Impfung mit Pasteur'schem Milzbrandvaccin getödtet war.

13	36—40° C.	2 Contr. sofort	406—945
"	"	2 nach 1 Std.	72—49
"	"	2 " 2 "	93—60
"	"	2 " 3 "	90—96
"	"	2 " 4 "	65—106
"	"	2 " 5 "	55—18

Tabelle XIV.

Versuche mit Milzbrandbacillen und Blut von immunen Hammeln.

14	37—38° C.	2 Contr. sofort	4576—4872
"	"	2 nach 1 Std.	1400—1650
"	"	2 " 2½ "	195—208
"	"	2 " 3 "	2360—2650
"	"	1 " 18 "	Unzählbar
"	"	1 " 20 "	"
15	39° C.	2 Contr. sofort	11466—12045
"	"	2 nach ½ Std.	2660—3194
"	"	2 " 1 "	1813—2000
"	"	1 " 2 "	1764
"	"	2 " 2½ "	427—660
"	"	1 " 6 "	8451

Tabelle XV.

Versuche mit Blut von Kaninchen und Milzbrandbacillen.
(Die Proben werden erst nach längerem Stehen geimpft).

Vers.-No.	Temp., bei welcher die Proben vor und nach der Impfung standen	Die Proben werden geimpft nach:	Platten werden angefertigt nach der Impfung:	Zahl der gewachsenen Colon.
15	38° C.	2 Std.	2 Contr. sofort	952—2060
"	"	"	2 nach 2 Std.	6—15
"	"	4 "	2 " 2 "	1—45
"	"	6 "	2 " 2 "	672—134
"	"	8 "	2 " 2 "	308—333
16	19—21° C.	2 Contr. sofort		7000—6930
"	"	1 "	1 nach 4 Std.	2
"	"	3 "	1 " 4 "	4
"	"	4 "	1 " 5½ "	46
"	"	1 "	1 " 22 "	97
"	"	3 "	1 " 20 "	175
"	"	4 "	1 " 20 "	246
17	Vor der Impfung bei 19—21° C., nach der Impfung bei 37·5° C.	2 Contr. sofort		7000—6930
"	"	3 "	1 nach 4 Std.	108
"	"	4 "	1 " 5½ "	62
"	"	3 "	1 " 20 "	Unzählbar
"	"	4 "	1 " 20 "	"

Tabelle XVI.

Versuche mit erhitztem Blut.

Vers.-No.	Thierspecies	Temperatur, auf welche das Blut erwärmt wurde.	Temperatur, bei welcher die Proben nach der Impfung standen	Platten werden angefertigt nach:	Zahl der gewachsen. Colonien
18	Hund	nicht erhitzt	37·5°	2 Contr.	925—987
"	"	"	"	2 nach 1 Std.	118—845
"	"	10 Minuten auf 58° C.	"	2 " 1 "	981—1005
"	"	30 Minuten auf 58° C.	"	2 " 1 "	1372—1470
19	Kaninchen	nicht erhitzt	"	2 Contr.	20—44
"	"	"	"	2 nach 1 Std.	0—0
"	"	"	"	2 " 2 "	0—0
"	"	"	"	2 " 3 "	0—0
"	"	"	"	2 " 4 "	0—0
"	"	"	"	2 " 17 "	0—0
"	"	45 Minuten auf 45° C.	"	2 Contr.	20—44
"	"	"	"	2 nach 1 Std.	32—60
"	"	"	"	" 2 "	77
"	"	"	"	" 3 "	20
"	"	"	"	" 4 "	172
"	"	"	"	" 16 "	Unzählig

Tabelle XVII.

Versuche mit einigen thierischen Flüssigkeiten.

Vers.-No.	Thierspecies	Art der Flüssigkeit	Temperatur, bei welcher die Proben nach der Impfung standen	Platten werden angefertigt nach:	Zahl der gewachsen. Colonien
20	Mensch	Pleuritisches Exsudat	37·5° C.	2 Contr.	282—320
"	"	"	"	2 nach 1 Std.	0—0
"	"	"	"	2 " 2 "	0—0—0
20	Hund	Humor aqueus	"	2 Contr.	925—987
19	"	Liquor pericard.	"	2 nach 1 Std.	294—320
"	"	"	"	2 " 1½ "	20—24
21	Kaninchen	Humor aqueus	"	2 Contr.	1029—1174
24	"	"	"	2 nach 2 Std.	1—0
"	"	Liquor pericard.	"	1 " 2 "	2
"	"	Humor aqueus	"	2 Contr.	20—0
"	"	"	"	2 nach 2 Std.	0—0

Tabelle XVIII.

Versuche mit Blut von Kaninchen und A. Bacillus subtilis.

Vers.-No.	Temperatur, bei welcher die Proben standen	Platten werden angefertigt nach:	Zahl der gewachsenen Colonien
25	—	2 Contr. sofort	12220—2246
"	38° C.	2 nach 2 Std.	0—0
22	—	2 Contr. sofort	308—320
"	37·5° C.	2 nach 2½ St.	0—0

B. Bacillus Megaterium.

26	—	2 Contr. sofort	1160—1320
"	38° C.	2 nach 2 Std.	31—154
20	—	2 Contr. sofort	1029—637
"	37·5° C.	2 nach 2½ St.	154—0

C. Staphylococcus pyogenes aureus.

27	—	2 Contr. sofort	9576—9460
"	37·5° C.	2 nach 2½ St.	10416—7660

TABLE 11

Experiments with anthrax bacilli and rabbit blood.

I. Experiment number -

II. Temperature at which the sample were left to stand after inoculation-

III. Period between inoculation and preparation of plates.

1.- 3 controls immediately.

2. 2 after 1 hour-

IV. Number of colonies grown

TABLE 12

Experiments with anthrax bacilli and mouse blood. (Headings as in Table 11)

1. Innumerable.

2. Experiment with anthrax bacilli and pigeon blood.

TABLE 13

Experiments with anthrax bacilli and blood of a non-immune sheep.

I. Experiment number -

II. Temperature at which the samples were left to stand after inoculation-

III. Period between inoculation and preparation plates.

1. 2 controls immediately.

2. 2 after 1 hours

IV. Number of colonies grown.

3. Innumerable

4. Experiments with blood of a sheep killed 48 hours after inoculation, with Pasteur anthrax vaccine I. --

TABLE 14

Experiments with anthrax bacilli and blood of immune sheep.

(Headings as in Table 13).

1. 2 controls immediately.

2. 2 after 1 hour.

TABLE 15.

Experiments with rabbit blood and anthrax bacilli. (the samples inoculated after being left to stand for some time).

I. Experiment number -

II. Temperature at which the samples were left to stand before and after inoculation.

III. Samples inoculated after:

IV. Plates prepared after inoculation:

1. 2 controls immediately.

2. 2 after 2 hours.

V. Number of colonies grown.

TABLE 16.

Experiments with heated blood.

I. Experiment number.

II. Animal species.

1. dog.

2. rabbit.

III. Temperature to which the blood was heated.

1. Not heated .

2. 10 minutes to 52° C

3. Not heated.

IV. Temperature at which the samples were left to stand after inoculation.

V. Plates prepared after :

1. 2 controls.

2. 2 after 1 hour.

VI. Number of colonies grown.

1. innumerable.

TABLE 17.

Experiments with a few animal fluids.

I. Experiment number .

II. Animal speicies.

1. Human.

2. dog.

3. rabbit

III. Nature of fluid.

1. Pleuritic exudate.

2. aqueous humor.

3. Pericardiac fluid.

IV. Temperature at which the samples were left to stand after inoculation.

V. Plates prepared after :

1. 2 controls.

2. 2 after 1 hour.

VI. Number of colonies grown.

TABLE 18.

Experiments with the blood of rabbits and A. Bacillus subtilis, B . Bacillus Megaterium, C. Staphylococcus pyogenes aureus..

I. Experiment number

II. Temperature at which the samples left to stand

III. Plates were prepared after :

1. 2 controls immediately.

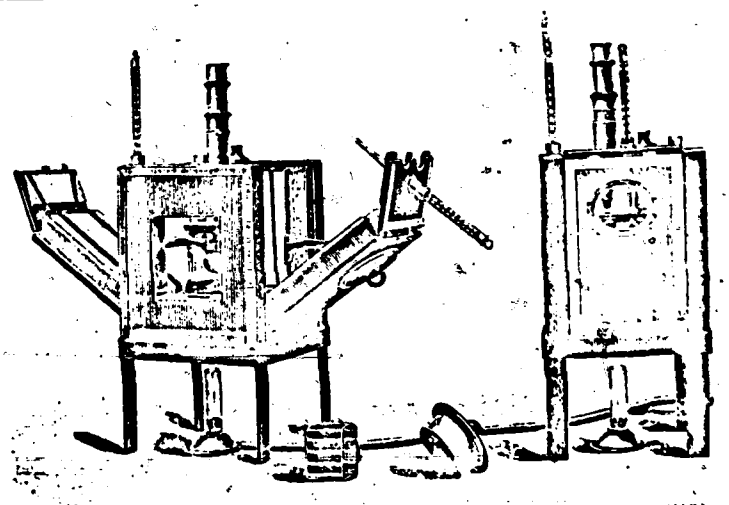
2. 2 after 2 hours.

IV. Number of colonies grown.

-o-o-o-o-o-

Figure on p. 373:

Heating chamber. Left, opened; frontal view. Right, closed, lateral view. Between the two illustrations the conical lid is shown on the surface of the table, for closure of the lateral opening, as well as a small rack with which seceral reserve preparations can be housed in the chamber.



Wärmekasten. Links geöffnet, Frontansicht; rechts geschlossen, Seitenansicht. Zwischen beiden Abbildungen auf der Tischfläche der conische Deckel zum Verschluss der seitlichen Oeffnung, sowie ein kleines Gestell, mit Hilfe dessen mehrere Reservepräparate im Kasten untergebracht werden können.